

EXHIBIT 1



US005304487A

United States Patent [19]
Wilding et al.

[11] **Patent Number:** 5,304,487
[45] **Date of Patent:** Apr. 19, 1994

[54] **FLUID HANDLING IN MESOSCALE ANALYTICAL DEVICES**

[75] **Inventors:** Peter Wilding, Paoli; Larry J. Kricka, Berwyn; Jay N. Zemel, Jenkintown, all of Pa.

[73] **Assignee:** Trustees of the University of Pennsylvania, Philadelphia, Pa.

[21] **Appl. No.:** 877,536

[22] **Filed:** May 1, 1992

[51] **Int. Cl.** G01N 31/00

[52] **U.S. Cl.** 435/291; 422/55; 422/58; 422/61; 435/7.2; 436/164; 436/524; 436/809

[58] **Field of Search** 422/55, 58, 61; 436/524, 164, 807, 809, 49, 501, 180; 435/2, 7.21, 359, 291, 6, 7.2

[56] **References Cited**

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Primary Examiner—James C. Housel

Assistant Examiner—Ramon Torres

Attorney, Agent, or Firm—Testa, Hurwitz & Thibault

[57] ABSTRACT

Devices are provided for analyzing a fluid cell containing sample. The devices comprise a solid substrate, microfabricated to define at least one sample inlet port and a mesoscale flow system. The mesoscale flow system includes a sample flow channel, extending from the inlet port, and a cell handling region for treating cells disposed in fluid communication with the flow channel. The devices may further include a structure inducing flow of cells in the sample through the flow system. In one embodiment, the cell-handling region may comprise a cell lysis structure to enable the lysis of cells in the sample, prior to, e.g., the detection of an intracellular component in the cell sample. In another embodiment, the cell handling region may comprise a cell capture region, comprising binding sites which reversibly bind to a specific population of cells in the cell sample, to permit the isolation of the specific cell population from the sample. The devices can be utilized in a wide range of automated sensitive and rapid tests for the analysis of a fluid cell containing sample.

26 Claims, 7 Drawing Sheets

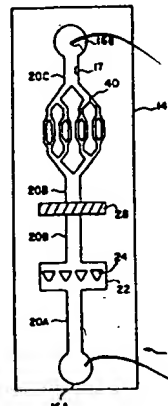


EXHIBIT 2



US005770029A

United States Patent [19]

[11] **Patent Number:** 5,770,029

Nelson et al.

[45] **Date of Patent:** Jun. 23, 1998

[54] **INTEGRATED ELECTROPHORETIC MICRODEVICES**

[75] **Inventors:** Robert J. Nelson, Alameda; Herbert H. Hooper, Belmont, both of Calif.; James Landers, Rochester, Minn.

[73] **Assignee:** Soane Biosciences, Hayward, Calif.

[21] **Appl. No.:** 690,307

[22] **Filed:** Jul. 30, 1996

[51] **Int. Cl.⁶** G01N 27/26; G01N 27/447

[52] **U.S. Cl.** 204/604; 204/453

[58] **Field of Search** 204/453, 604

[56] **References Cited**

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Primary Examiner—Kathryn L. Gorgos

Assistant Examiner—John S. Starsiak, Jr.

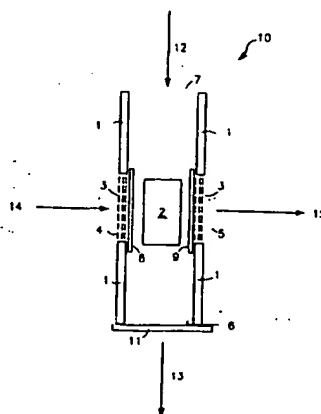
Attorney, Agent, or Firm—Flehr Hohbach Test Albritton & Herbert LLP

[57]

ABSTRACT

Integrated electrophoretic microdevices comprising at least an enrichment channel and a main electrophoretic flowpath are provided. In the subject integrated devices, the enrichment channel and the main electrophoretic flowpath are positioned so that waste fluid flows away from said main electrophoretic flowpath through a discharge outlet. The subject devices find use in a variety of electrophoretic applications, including clinical assays.

14 Claims, 5 Drawing Sheets



United States Patent [19]

Soane et al.

US005126022A

[11] Patent Number: 5,126,022

[45] Date of Patent: Jun. 30, 1992

[54] METHOD AND DEVICE FOR MOVING MOLECULES BY THE APPLICATION OF A PLURALITY OF ELECTRICAL FIELDS

[75] Inventors: David S. Soane; Zoya M. Soane, both of Piedmont, Calif.

[73] Assignee: Soane Technologies, Inc., Hayward, Calif.

[21] Appl. No.: 487,021

[22] Filed: Feb. 28, 1990

[51] Int. Cl. G01N 27/26

[52] U.S. Cl. 204/183.1; 204/299 R; 204/300 R

[58] Field of Search 204/182.8, 299 R, 183.1, 204/300 R, 180.1

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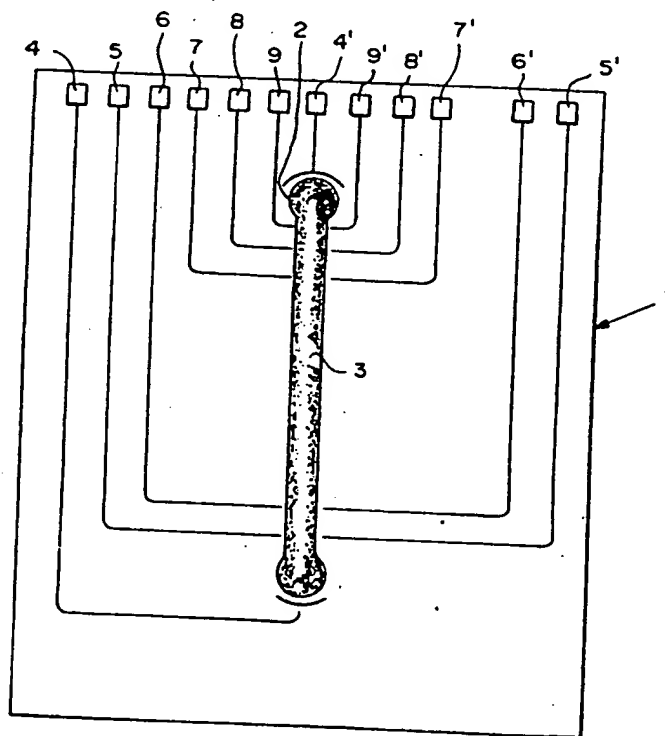
0356187 2/1990 European Pat. Off.
WO84/02001 5/1984 PCT Int'l Appl.

Primary Examiner—John Niebling
Assistant Examiner—David G. Ryser
Attorney, Agent, or Firm—Kilpatrick & Cody

[57] ABSTRACT

Devices and methods are disclosed for moving charged molecules through a medium by the application of a plurality of electrical fields of sufficient strength and applied for sufficient amounts of time so as to move the charged molecules through the medium. The devices although preferably small in size, preferably generate large numbers (100 or more) of electrical fields to a movement area which preferably contains a liquid buffered or gel medium. Mixtures of charged molecules are pulled through the gel by the force of the electrical fields. The fields are preferably activated simultaneously or sequentially one after another at various speeds to create complex force field distributions or moving field waves along the separation medium. Charged molecules capable of moving quickly through the gel will be moved along by the faster moving field waves and be separated from slower moving molecules. The fields can be activated by computer software and can be used to move molecules away from and toward each other to obtain rapid and complex chemical synthesis, sequencing or reaction protocols.

21 Claims, 1 Drawing Sheet



United States Patent [19]

Sassi et al.



US005631337A

[11] Patent Number: 5,631,337
 [45] Date of Patent: May 20, 1997

[54] **THERMOREVERSIBLE HYDROGELS
 COMPRISING LINEAR COPOLYMERS AND
 THEIR USE IN ELECTROPHORESIS**

[75] Inventors: Alexander P. Sassi, Berkeley; Shi Lin, Fremont; M. Goretty Alonso-Amigo, Santa Clara; Herbert H. Hooper, Belmont, all of Calif.

[73] Assignee: Soane BioScience, Hayward, Calif.

[21] Appl. No.: 589,026

[22] Filed: Jan. 19, 1996

[51] Int. Cl.⁶ C08F 220/56; C08F 222/38

[52] U.S. Cl. 526/307.2; 526/306; 526/307.3;
 526/310

[58] Field of Search 526/306, 307.2,
 526/307.3, 310

[56] References Cited

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Primary Examiner—Tae Yoon

Attorney, Agent, or Firm—Flehr Hohbach Test Albritton & Herbert LLP

[57]

ABSTRACT

Thermoreversible hydrogels comprising non-ionic, linear copolymers, and methods of their use in electrophoresis, are provided. The subject copolymers comprise polyacrylamide backbones, where a portion of the acrylamide monomeric units compose hydrogen bonding groups as N-substituents. Combination of the subject copolymers with an aqueous phase provides thermoreversible hydrogels which find use as separation media in electrophoretic applications.

18 Claims, No Drawings



US005569364A

United States Patent [19]

Hooper et al.

[11] Patent Number: 5,569,364

[45] Date of Patent: Oct. 29, 1996

[54] SEPARATION MEDIA FOR ELECTROPHORESIS

[75] Inventors: Herbert H. Hooper, Belmont; Stephen Pacetti, Sunnyvale; David S. Soane, Piedmont, all of Calif.; Young C. Bae, Seoul, Rep. of Korea

[73] Assignee: Soane Biosciences, Inc., Hayward, Calif.

[21] Appl. No.: 241,048

[22] Filed: May 10, 1994

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 971,956, Nov. 5, 1992, abandoned.

[51] Int. Cl.⁶ C25B 9/00

[52] U.S. Cl. 204/455; 204/462; 204/466; 204/468; 204/605; 204/613; 204/616; 252/315.1; 526/303.1; 526/306; 524/555

[58] Field of Search 204/182.8, 299 R; 252/315.1; 526/303.1, 306; 524/555

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Primary Examiner—John Niebling

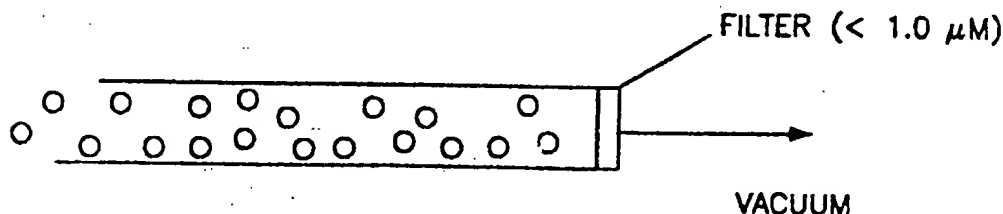
Assistant Examiner—C. Delacroix-Muirheid

Attorney, Agent, or Firm—Bertram I. Rowland, Ph.D.

[57] ABSTRACT

Separation media for electrophoresis, and methods of filling and flushing of electrophoretic devices such as capillaries are described. By preparing submicron to above-micron sized cross-linked gel particles and using gel swelling equilibrium concepts, such devices can be easily filled and flushed. Gel particles can be prepared by inverse suspension, precipitation and suspension polymerization. These particles can be swollen and collapsed by small changes in temperature, pH, and ionic strength of solvent. Other approaches involve the formation of reversible cross-links by use of polyelectrolyte complexes, chelating agents or copolymers of hydrophobic and hydrophilic repeat units. Finally, reversibly solubilized systems may be used to change the viscosity of the media.

24 Claims, 13 Drawing Sheets





US005750015A

United States Patent [19]

Soane et al.

[11] Patent Number: 5,750,015
[45] Date of Patent: May 12, 1998

- [54] **METHOD AND DEVICE FOR MOVING MOLECULES BY THE APPLICATION OF A PLURALITY OF ELECTRICAL FIELDS**
- [75] Inventors: David S. Soane; Zoya M. Soane, both of Piedmont, Calif.
- [73] Assignee: Soane Biosciences, Hayward, Calif.
- [21] Appl. No.: 615,642
- [22] Filed: Mar. 13, 1996

Related U.S. Application Data

- [63] Continuation-in-part of Ser. No. 430,134, Apr. 26, 1995, abandoned, which is a continuation of Ser. No. 196,763, Feb. 14, 1994, abandoned, which is a continuation of Ser. No. 880,187, May 7, 1992, abandoned, which is a continuation of Ser. No. 487,021, Feb. 28, 1990, Pat. No. 5,126,022.
- [51] Int. Cl.⁶ G01N 27/26; G01N 27/447
- [52] U.S. Cl. 204/454; 204/451; 204/547; 204/601; 204/643
- [58] Field of Search 204/547, 643, 204/454, 451, 601

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Primary Examiner—Kathryn L. Gorgos

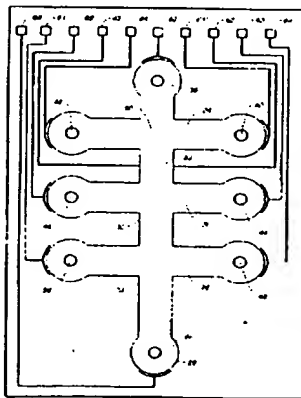
Assistant Examiner—John S. Starsiak, Jr.

Attorney, Agent, or Firm—Flehr Hohbach Test Albritton & Herbert LLP; Richard F. Trecartin

[57] ABSTRACT

Devices and methods are disclosed for moving charged molecules through a medium by the application of a plurality of electrical fields of sufficient strength and applied for sufficient amounts of time so as to move the charged molecules through the medium. The devices although preferably small in size, preferably generate large numbers (100 or more) of electrical fields to a movement area which preferably contains a liquid buffered or gel medium. Mixtures of charged molecules are pulled through the gel by the force of the electrical fields. The fields are preferably activated simultaneously or sequentially one after another at various speeds to create complex force field distributions or moving field waves along the separation medium. Charged molecules capable of moving quickly through the gel will be moved along by the faster moving field waves and be separated from slower moving molecules. The fields can be activated by computer software and can be used to move molecules away from and toward each other to obtain rapid and complex chemical synthesis, sequencing or reaction protocols.

47 Claims, 2 Drawing Sheets



United States Patent [19]

Sane



US005135627A

[11] Patent Number: 5,135,627

[45] Date of Patent: Aug. 4, 1992

[54] MOSAIC MICROCOLUMNS, SLABS, AND SEPARATION MEDIA FOR ELECTROPHORESIS AND CHROMATOGRAPHY

[75] Inventor: David S. Soane, Piedmont, Calif.

[73] Assignee: Soane Technologies, Inc., Hayward, Calif.

[21] Appl. No.: 597,528

[22] Filed: Oct. 15, 1990

[51] Int. Cl.⁵ C25B 1/00; B01D 57/02; B01D 61/42

[52] U.S. Cl. 204/182.8; 204/180.1; 204/299 R; 428/327

[58] Field of Search 204/182.8, 180.1, 299 R; 428/327

[56] References Cited

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Primary Examiner—John Niebling
Assistant Examiner—Caroline Koestner
Attorney, Agent, or Firm—Kilpatrick & Cody

[57] ABSTRACT

A method and compositions for separating molecules based on molecular size, shape, affinity, chirality, weight, charge, and hydrogen bonding, using a mosaic matrix formed by polymerizing a dispersion of dispersoids within a polymeric matrix. The dispersoids and matrix can be of the same or different hydrophobicity or hydrophilicity. The dispersoids can be porous or non-porous. The mosaic matrix can be used with existing chromatographic and electrophoresis apparatus to effect an enhanced separation of molecules, particularly of nucleic acids and peptides, by application of a solution and/or an electrical field to the matrix. The solution can form a pH, ionic, or composition gradient, and be applied using gravity or under pressure. The electrical field can be continuous, pulsed, or two-dimensional.

33 Claims, 1 Drawing Sheet

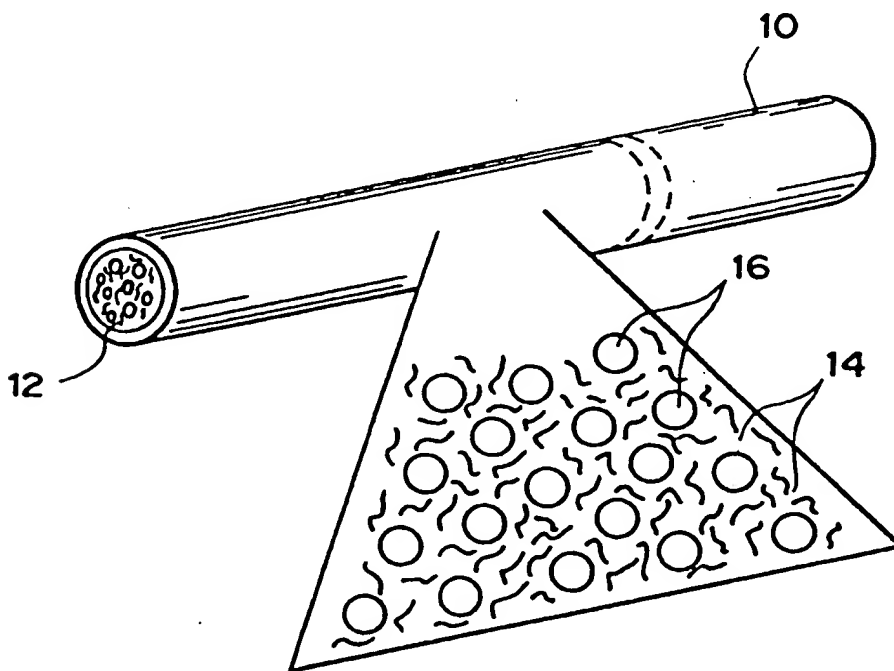


EXHIBIT 8



US005498392A

United States Patent [19]
Wilding et al.

[11] **Patent Number:** 5,498,392
[45] **Date of Patent:** Mar. 12, 1996

[54] **MESOSCALE POLYNUCLEOTIDE
AMPLIFICATION DEVICE AND METHOD**
[75] **Inventors:** Peter Wilding, Paoli; Larry J. Kricka,
Berwyn, both of Pa.

[73] **Assignee:** Trustees of the University of
Pennsylvania, Philadelphia, Pa.

[21] **Appl. No.:** 308,199

[22] **Filed:** Sep. 19, 1994

Related U.S. Application Data

- [63] Continuation of Ser. No. 877,662, May 1, 1992, abandoned.
[51] **Int. Cl.⁶** G01N 25/00; G01N 33/50;
C12P 19/34
[52] **U.S. Cl.** 422/68.1; 422/50; 422/55;
422/61; 422/62; 422/63; 422/82.05; 422/102;
435/6; 435/91.1; 435/91.2; 435/285.1; 435/285.2;
435/810; 436/807; 935/78; 935/88
[58] **Field of Search** 435/6, 91.1, 91.2,
435/288, 291, 316, 803, 810; 436/807;
536/22.1, 23.1, 24.1; 935/78, 88; 422/50,
55, 61, 62, 63, 68.1, 82.05, 102

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4,908,112	3/1990	Pace	204/299 R
4,911,782	3/1990	Brown	156/633
4,963,498	10/1990	Hillman et al.	436/69
5,135,720	8/1992	Uchida	422/107
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(List continued on next page.)

Primary Examiner—W. Gary Jones
Assistant Examiner—Ardin H. Marschel
Attorney, Agent, or Firm—Dann, Dorfman, Herrell and Skillman

[57] ABSTRACT

Disclosed are devices for amplifying a preselected polynucleotide in a sample by conducting a polynucleotide polymerization reaction. The devices comprise a substrate microfabricated to define a sample inlet port and a mesoscale flow system, which extends from the inlet port. The mesoscale flow system includes a polynucleotide polymerization reaction chamber in fluid communication with the inlet port which is provided with reagents required for polymerization and amplification of a preselected polynucleotide. In one embodiment the devices may be utilized to implement a polymerase chain reaction (PCR) in the reaction chamber (PCR chamber). The PCR chamber is provided with the sample polynucleotide, polymerase, nucleoside triphosphates, primers and other reagents required for the polymerase chain reaction, and the device is provided with means for thermally controlling the temperature of the contents of the reaction chamber at a temperature controlled to dehybridize double stranded polynucleotide, to anneal the primers, and to polymerize and amplify the polynucleotide.

27 Claims, 11 Drawing Sheets

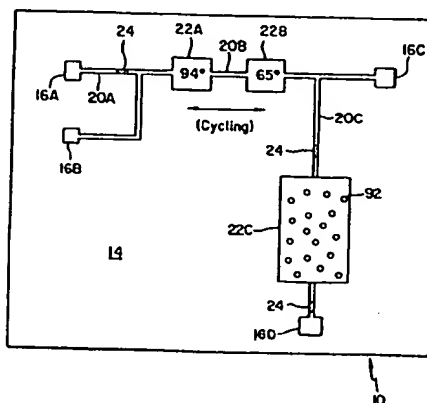


EXHIBIT 9

US005587128A

United States Patent [19]
Wilding et al.

[11] Patent Number: 5,587,128
[45] Date of Patent: *Dec. 24, 1996

[54] MESOSCALE POLYNUCLEOTIDE
AMPLIFICATION DEVICES

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Berwyn, both of Pa.

[73] Assignee: The Trustees of the University of
Pennsylvania, Philadelphia, Pa.

[*] Notice: The term of this patent shall not extend
beyond the expiration date of Pat. No.
5,304,487.

[21] Appl. No.: 338,728

[22] Filed: Nov. 14, 1994

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 308,199, Sep. 19, 1994, Pat.
No. 5,498,392, which is a continuation of Ser. No. 877,662,
May 1, 1992, abandoned.

[51] Int. Cl.⁶ C12Q 1/68; G01N 33/50;
G01N 21/01

[52] U.S. Cl. 422/50; 422/54; 422/55;
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422/82.01; 422/82.02; 422/82.05; 422/82.06;
422/82.07; 422/82.08; 422/82.09; 422/129;
422/131; 422/138; 435/6; 435/90; 435/91.1;
435/91.2; 435/91.3; 435/91.5; 435/91.51;
435/173.1; 435/174; 435/176; 435/177;
435/283.1; 435/285.1; 435/285.2; 435/287.1;
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435/287.9; 435/288.7; 435/289.1; 435/290.1;
435/292.1; 435/299.1; 435/808; 435/810;
435/814; 436/518; 436/524; 436/525; 436/528;
436/531; 436/532; 436/535; 436/63; 436/164;
436/165; 436/166; 436/169; 436/172; 436/175;
437/1; 437/5; 437/51; 437/61; 437/180;
437/181; 437/189; 437/225; 437/946; 536/22.1;
536/23.1; 935/77; 935/78; 935/88

[58] Field of Search 422/50, 54, 55,
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6, 5, 291, 810, 90, 91, 91.3, 91.5, 91.51,
173.1, 174, 176, 177, 283.1, 285.1, 285.2,
287.1-3, 287.7-9, 288.7, 289.1, 290.1,
292.1, 299.1, 808, 814; 437/1, 5, 51, 61,
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25.3; 935/77, 78, 88; 436/518, 524, 525,
528, 531, 532, 535, 63, 164-6, 169, 172,
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Skillman

[57]

ABSTRACT

Disclosed are devices for amplifying a preselected poly-
nucleotide in a sample by conducting a polynucleotide
amplification reaction. The devices are provided with a
substrate microfabricated to include a polynucleotide ampli-
fication reaction chamber, having at least one cross-sectional
dimension of about 0.1 to 1000 μ m. The device also includes
at least one port in fluid communication with the reaction
chamber, for introducing a sample to the chamber, for

(Abstract continued on next page.)

